In Situ Mineralized Nanocellulose-Alginate Bioink System for Injectable Bone Graft/3D Printing Applications

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Introduction

Biopolymer-Mineral composite systems that can be both injected and bioprinted, have great potential for clinical applications in bone tissue engineering. The calcium phosphate ink systems used for 3D printing purposes can't be used to suspend live cells as the solid and stiff matrix will destroy the cells while printing and limit diffusion of nutrients during culture. The goal of the present study was to develop a bone-mimetic bioink where hydroxyapatite (HA) particles are synthesized in situ in nanocellulose (NC) solution, using the nanocellulose fibers as a HA nucleation template thus developing a bone like Cellulose-HA nanocomposite system. The hypothesis was that HA synthesis within a nanocellulose-alginate matrix would reduce shear on encapsulated cells during printing/injecting while facilitating nutrient exchange post printing.

Methods

Nanocellulose was prepared from reagent grade cellulose in 1M NaOH (3% w/v) using High Intensity Ultra-Sonication (HIUS) for 20 minutes at ~1Hz to prepare cellulose nanofibrills (CNF). 3% nanocellulose solution was used to prepare 125mM of $Ca(OH)_2$. The same volume of H3PO4 (75mM) was added dropwise while stirring at 700 rpm over 24 hr for HA nucleation and crystallization. Sodium citrate (10% w/v) was used as a dispersant during the reaction. The mineralized nanocellulose solution was then mixed with a 25% Alginate (ALG) hydrogel. SEM, XRD and ATR-FTIR were performed to analyze the nanocomposite formation between HA and Nanocellulose. An AR500 (TA Instruments) rheometer was used to analyze the viscosity, printability and injectability. Compressive mechanical properties of the formulations in un-crosslinked and in those crosslinked with CaCl2 (100 mM) were tested using an Instron 5969 testing system with a 1kN load cell at a crosshead speed of 1mm/min. A CELLINK BioX 3D bioprinter was used to print the Nanocellulose-Alginate-HA composite with/without human mesenchymal stem cells (hMSCs), crosslinked with 100mM CaCl2, in a 1×1×0.3 cm, 4 layered structure with a 410 µm plastic nozzle at 6 mm/s speed and 200 kPa pressure. For degradation studies, the printed structures (without cells) were incubated in DMEM media for 28 days. Cell viability was analyzed by live and dead cell assay till day 28.

Results

The formation of HA crystals using nanocellulose as template for crystallization was observed with SEM (Fig 1).

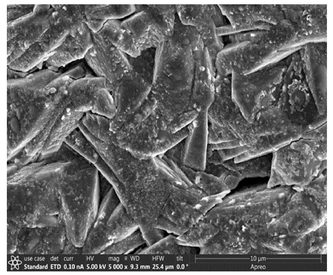

Figure 1. HA crystal formation over NC polymer chains to form a composite material system.

Peaks for PO43− at 564 cm−1, 603 cm−1 in the ATR-FTIR analysis indicate HA crystallization which was enhanced by the addition of nanocellulose (Fig 2), while alginate had no effect. The 211 peak in the XRD spectra confirmed the formation of crystallized HA deposits with nanocellulose (data not shown).

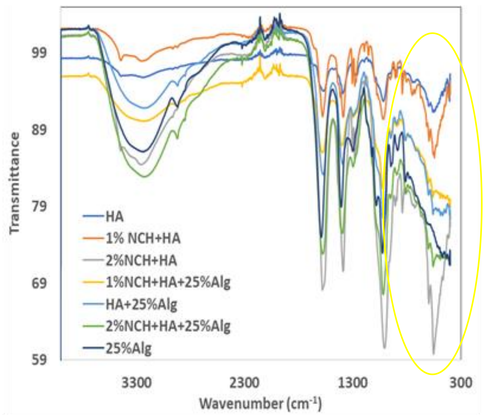

Figure 2. ATR-FTIR spectra showing HA crystal peaks formed NC.

Rheological testing demonstrated the shear thinning behavior in all the tested groups, which is a prerequisite for 3D printing (Fig. 3A). The mechanical testing studies demonstrated that the nanocellulose-HA composite with alginate had a significantly (*p>0.01) higher elastic modulus than both HA crystallized with alginate and alginate alone (Fig. 3B).

Results

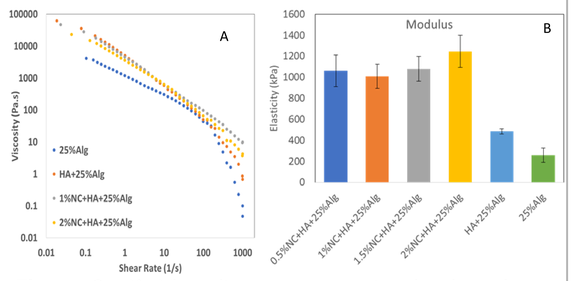

Figure 3. A) Viscosity measurements showing shear thinning behavior of HA-NC-Alginate bioink. Compressive modulus of NC-HA-Alginate composite was the highest.

The injectability test further demonstrated that the NC-HA-ALG bioink could regain its rheological properties within 5 minutes of injection and thus could be printed as standalone structures (Fig. 4).

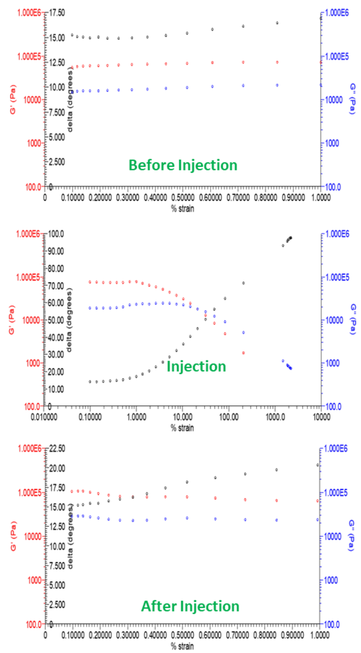

Figure 4. Injectability testing studies demonstrating that the of NC-HA-Alginate Bioink system is both injectable and 3D printable

Results

The 2%NC-25%ALG-HA was thus chosen for degradation study and was found to stable without any significant change in dry mass (Fig. 5).

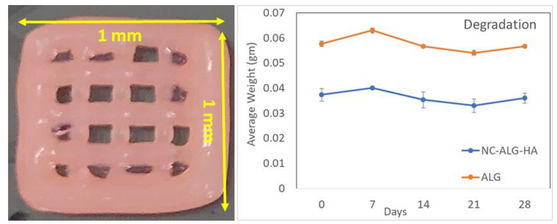

Figure 5. Degradation studies of 3D printed NC-HA-Alginate structures over 28 days showed no degradation.

The cell culture studies demonstrated viable cells (hMSCs) after printing as well as the ability of the system to sustain them for 28 days under in vitro culture conditions (Fig. 6).

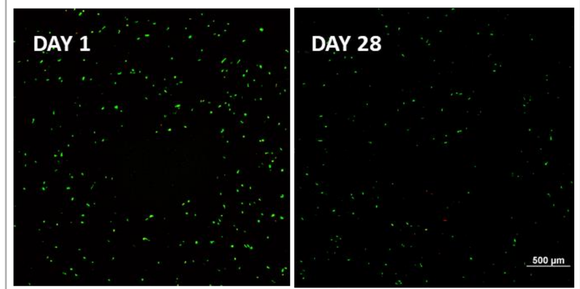

Figure 6. Live/ Dead cell assay demonstrating cell viability of hMSCs over a period of 28 days. The NC-HA-Alginate bioink system can print viable cells without damaging them with the shear pressure created by HA particles.

Conclusion

The current system can potentially promote regeneration of bone tissue while providing mechanical support after injury. It can be injected through a minimal invasive surgery into complex, 3D voids, thus providing a potential strategy for treatment of critical sized bone defects.